

Epigallocatechin gallate content change of the fresh tea leaf homogenates extracted by different methods in extraction and preservation

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Abstract: The fresh leaves of China green tea, *Camellia sinensis*, were collected from Fuyang, Zhejiang Province, China, in April. The tea polyphenols was extracted by four different methods (homogenized with distilled water at room temperature, homogenized with 0.3% citric acid (w/v) at room temperature, 5- min boiling and homogenized with distilled water at room temperature, homogenized with 85°C distilled water), and after preserving at room temperature, the change of the Epigallocatechin gallate (EGCG) contents of the extracts was investigated. Results indicated that the EGCG content of homogenate extracted with 85°C distilled water was the highest before the extract was preserved, followed by that of the extract homogenized with 0.3% citric acid at room temperature. During preservation, EGCG content changed obviously. The EGCG contents of homogenates extracted with distilled water at room temperature and 85°C distilled water declined quickly and separately reduced to 21.52% and 54.6% of their initial contents after preservation for 12 h. The EGCG contents extracted by 0.3% citric acid (w/v) solvent at room temperature and 5- min boiling/homogenized with distilled water at room temperature declined relatively slowly ,and separately reduced to 76.9% and 85.16% of their initial contents after preservation for 12 h. It was also found that the citric acid can prevent the degradation of EGCG and the extract solution color is light green

Key words: Epigallocatechin gallate (EGCG); Homogenate extraction; Content change

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Introduction

Tea polyphenols, extracted from tea leaves, is a hydroxyl phenol compound. Of the components of green tea (*Camellia sinensis*), the content of (−)-epigallocatechin gallate (EGCG) is high, and this catechin has been extensively studied (Harold 1992). Some epidemiological and animal studies revealed that EGCG had a protective effect against various cancer, such as lung, prostate, and breast cancers. Laminin, one of the major glycoproteins of membrane basilaris, has multiple biological activities which are mediated by interaction with specific cell membrane receptors. Latest research indicated that anticancer action of EGCG was mediated by the 67-kDa laminin receptor (Hirofumi *et al.* 2004; Garbisa *et al.* 1999, Kim and Moon 2005; Roomi *et al.* 2005; Cao *et al.* 1999; Bachmeier *et al.* 2005). EGCG has both antimatrix metalloproteinase and antiangiogenesis activities and can prevent the formation of solid tumors. In the extraction and preservation process, degradation of EGCG is very fast. Degenerated enzyme activity, pH and temperature are also the significant affecting factors on degradation of EGCG. By adjusting these factors, degradation of EGCG can be prevented or delayed in process of extracting fresh tea leaves.

In the present study, we measured the EGCG contents of China green tea using different extracting methods during the preservation process and attempted to develop the optimal methods for the extraction and preservation of EGCG (Yuko Yoshida *et al.* 1999; Peterson *et al.* 2004; Vaishali Sharma *et al.* 2005; Wang *et al.* 2004).

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Material and method

Chemicals and reagents

Acetonitrile, ethyl acetate, ethanol, citric acid were provided by Beijing Beihua Fine Chemicals Co., Ltd; sulfuric acid was from Dima Technology Inc. (USA); epigallocatechin gallate was from Sigma Chemical Co. All chemicals used in this study were analytical grade or HPLC grade.

Samples

Fresh tea leaves were sampled from Fuyang, Zhejiang Province, China. All samples were stored at −4°C for further use.

Homogenate extraction of fresh tea leaves

Four different methods were used to treat the samples. Method 1: fresh tea leaves was homogenized with distilled water at room temperature; Method 2: fresh tea leaves homogenized with 0.3% citric acid (w/v) at room temperature; Method 3: fresh tea leaves was boiled for 5 min to kill enzymes and homogenized with distilled water at room temperature; Method 4: fresh tea leaves was homogenized with 85°C distilled water (Fig. 1).

Fresh tea leaves (20 g) were homogenized with 200 mL solvent by high-speed homogenate machine for some times. The pulp was filtrated under the condition of reduced pressure to obtain extracting solutions.

All four kinds of homogenized extracts were preserved at room temperature. The contents of EGCG were determined by HPLC for different homogenate and preservation time.

HPLC analysis

The analysis was performed with a Hypersil C₁₈ (ODS) (5 μm, 4.6×250 mm), with the eluent of 85.8% acetonitrile, 12% water, 2% ethyl acetate, 0.2% sulfuric acid, and flow rate was 1.0 mL·min⁻¹ (Jasco 1580 HPLC Pump) and monitored by a Jasco 1575 variable wavelength detector, detected at 280 nm. All solvents (HPLC Grade) and samples were filtered through a 0.45 μm filter.

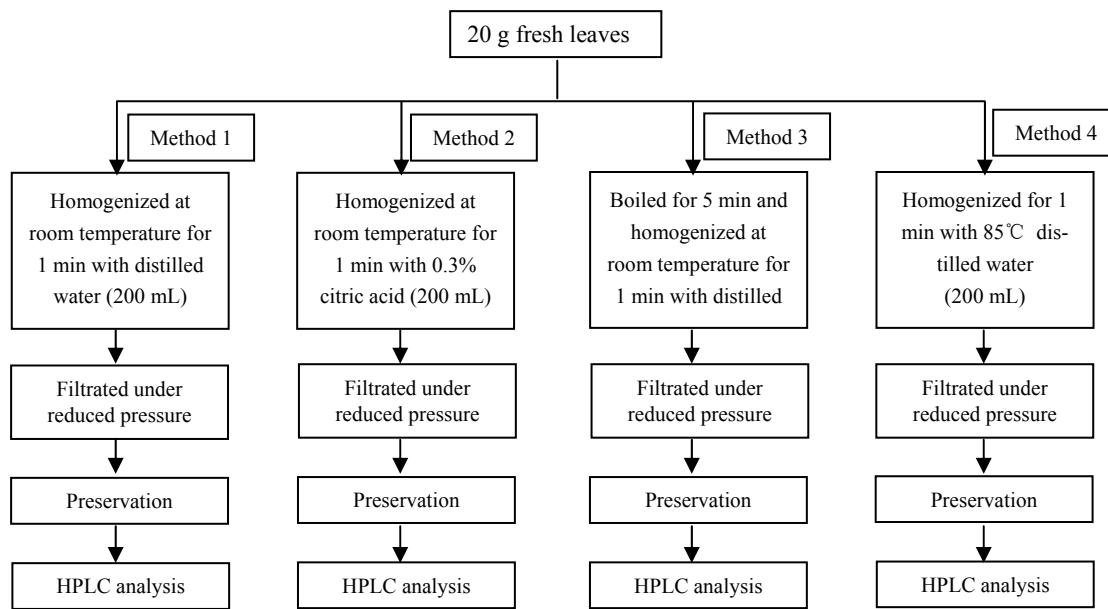


Fig. 1 Technological processes of homogenate extraction of fresh tea leaves

Results and analysis

Change tendency of EGCG contents during homogenate extraction

The change tendencies of EGCG contents of the fresh tea leaf homogenate extracted by the four methods are shown in Fig. 2. The optimal homogenate extracting time is 1 min for all the extracting methods. Under such condition, the EGCG content in the extract homogenized with 85°C distilled water (Method 4) is the highest, followed by that of the extract homogenized with 0.3% citric acid at room temperature (Method 3). These may be attributed to high extraction temperature and the additional of citric acid. The EGCG contents of the extract by the other two kinds of methods are similar and lower than those of the former two. As time progressing, the EGCG contents of fresh tea leaf homogenate extracted by Method 1 and Method 4 decrease rapidly and the decrease tendencies are faster than those by Method 2 and Method 3 (Fig. 2).

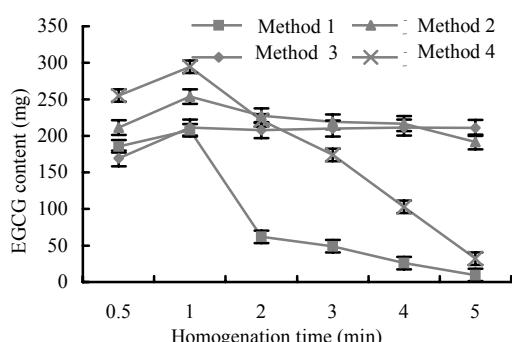


Fig. 2 EGCG content against homogenate time

Changes of the solution color and EGCG content after preservation

Change of the solution color

Degradation of EGCG can be also identified from the change of the solution color. The higher degradation degree, the deeper the color is. The solution color before preservation and after preserved for 12 h were compared for the four homogenate extracts (Table 1). The initial extract solution color by Method 2 was the lightest and the color by Method 4 was the darkest. After preservation for 12 h, the extract solution color by Method 4 was the darkest, followed by Method 1, and the extract solutions by Method 2 and Method 3 had no obviously change. The longer the preservation time, the deeper is the color. When the degradation degree is low, the change of solution color is not obvious.

Table 1. The change of solution color before and after preservation

	Method 1	Method 2	Method 3	Method 4
Before preservation	Yellowish green	Light yellowish green	Yellowish green	Light brown
After preservation	Brown	Light deepen yellowish green	Deepen yellowish green	Light deepen brown

Change of the EGCG content in the preservation process

In preservation process, the change of EGCG content of homogenate is obvious (Fig. 3, Fig. 4). The EGCG contents of homogenates extracted by Method 1 and Method 4 reduced quickly. After preserved for 12 h, the EGCG contents of homogenates extracted by Method 1 and Method 4 declined to 21.52% and 54.6%, respectively, of their initial contents. Compared to those by Method 1 Method 4, the EGCG contents of homogenates extracted by Method 2 and Method 3 declined relatively slowly, and, down to 76.9% and 85.16% of their initial contents, respectively, after 12-h preservation.

At anytime of the preservation process, the EGCG content of

homogenate extract by Method 1 was lower than those of the other three kinds of homogenate extracts. After preserved for 12 h, the EGCG content of the extract by Method 1 was still nearly 3 times lower than those of the extracts by other three kinds of methods

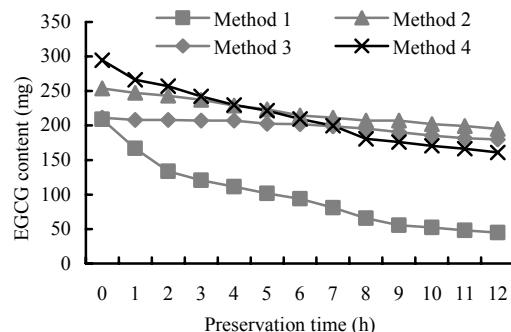


Fig. 3 EGCG contents of homogenate extracts at the different preservation time

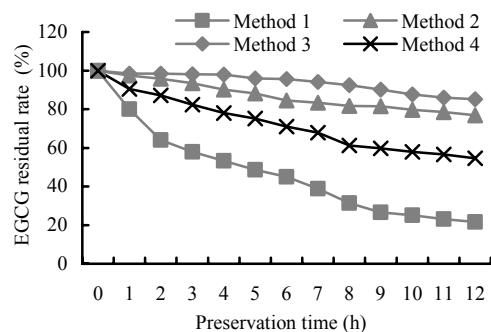


Fig. 4 EGCG residual ratios of homogenate extracts at the different preservation time

Discussion

Fresh material

The raw material used in this experiment is fresh tea leaves, which have high water content. The pit channel of leaf cell maintains in good unimpeded and permeable conditions, which cause the cell instantaneous smash through the high-speed homogenate machine. Water-soluble secondary metabolites in the tea cell, like EGCG, are quickly dissolved and extracted instantaneously.

Temperature

The results showed that the extraction temperature was a remarkable affecting factor for the change of EGCG content. High temperature accelerates molecular transport and matter diffusion of the system, thus increasing the dissolution speed of EGCG. When the sample is treated with instantaneous high temperature, degeneration enzymes of fresh leaves are destroyed partly.

Citric acid-depressor

In the preservation process, as time progressed, EGCG content still had a little decline. The reason lies in the activity of EGCG degradation enzymes is destroyed incompletely. At the same extraction condition after adding citric acid into the extract solvent, the EGCG content is higher than that in the no- citric acid solvent. These results indicate that citric acid has a certain effect

on the activity of degeneration enzymes.

The method and extraction efficiency of catechins from tea are critical in further study on the functionality of these substances. In general, organic solvents such as methanol and acetonitrile have been used as solvents to quantitatively extract catechins from tea leaves. This method is very useful for the measurement of catechins in leaves; however, for studying people consumption for tea, extraction by using these organic solvents may not reflect actual levels of the catechins in the tea beverage. Some studies on the extraction condition of tea using hot water were reported. However, the purpose of these studies was to provide high quantitative information, and wasted a large quantity of energy. In this study, we found that citric acid can prevent the degradation of EGCG and the extract solution color was light green. It can provide best condition for sensory evaluation of tea quality by color, flavor, or to survey the taste qualities of tea or to make most tasty teas and high quantitative of EGCG.

Overall, extracting EGCG by these extract methods in this study has low cost and high yields. This study should be particularly applied in commercial scales that require high quality and yields of product and short process cycle.

References

- Bachmeier, B.E., Iancu, C.M., Jochum, M., et al. 2005. Matrix metalloproteinases in cancer: comparison of known and novel aspects of their inhibition as a therapeutic approach [J]. Expert. Rev. Anticancer Ther., **5**(1): 149–163.
- Cao Yihai, Cao Renhai. 1999. Angiogenesis inhibited by drinking tea [J]. Nature, **398**: 381
- Garbisa, S., Biggin, S., Cavallarin, N., et al. 1999. Tumor invasion: molecular shears blunted by green tea [J]. Nature Medicine, **5**: 1216
- Harold, N. Graham. 1992. Green tea composition, consumption and polyphenol chemistry [J]. Preventive Medicine, **21**(3): 334–350
- Hirofumi Tachibana, Kiyoshi Koga, Yoshinori Fujimura, et al. 2004. A receptor for green tea polyphenol EGCG [J]. Nature, **11**(4): 380–381
- Kai On Chu, Chi Chiu Wang, Michael Scott Rogers, et al. 2004. Determination of catechins and catechin gallates in biological fluids by HPLC with coulometric array detection and solid phase extraction [J]. Analytica Chimica Acta, **510**(1): 69–76.
- Kim, C.H., Moon, S.K. 2005. Epigallocatechin-3-gallate causes the p21/WAF1-mediated G (1)-phase arrest of cell cycle and inhibits matrix metalloproteinase-9 expression in TNF-alpha-induced vascular smooth muscle cells [J]. Arch Biochem Biophys, **435**(2): 264–272.
- Peterson, J., Dwyer, J., Jacques, P., et al. 2004. Tea variety and brewing techniques influence flavonoid content of black tea [J]. Journal of Food Composition and Analysis, **17**(3–4): 397–405
- Roomi, M.W., Ivanov, V., Kalinovsky, T., et al. 2005. In vivo antitumor effect of ascorbic acid, lysine, proline and green tea extract on human colon cancer cell HCT 116 xenografts in nude mice: evaluation of tumor growth and immunohistochemistry [J]. Oncol Rep, **13**(3): 421–425.
- Vaishali Sharma, Ashu Gulati, S.D. Ravindranath, et al. 1999. A simple and convenient method for analysis of tea biochemical's by reverse phase HPLC [J]. Journal of Food Composition and Analysis, **18**: 583–594.
- Vaishali Sharma, Ashu Gulati, S.D. Ravindranath. 2005. Extractability of tea catechins as a function of manufacture procedure and temperature of infusion [J]. Food Chemistry, **93**(1): 141–148.
- Wang, R., Zhou, W. 2004. Stability of tea catechins in the bread making process [J]. J Agric Food Chem, **52**(26): 8224–8229.
- Yuko Yoshida, Masaaki Kiso and Tetsuhisa Goto. 1999. Efficiency of the extraction of catechins from green tea [J]. Food Chemistry, **67**(4): 429–433